DOCKET NO.: 2300.0202 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

Graeme I. Bell, et al.

Serial No.: 08/837,009 Group No.: 1804

Filed: April 11, 1997 Examiner: Not Yet Assigned

For: PREPRO INSULIN-LIKE GROWTH FACTORS I AND II

I, **Francis A. Paintin**, Registration No. **19,386** certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On October 2, 1997

Francis A. Paintin

Francis A. Paintin Reg. No. **19,386**

Box Missing Parts
Assistant Commissioner for Patents
Washington DC 20231

Sir:

RESPONSE TO NOTICE TO FILE MISSING PARTS OF APPLICATION

In response to the "Notice to File Missing Parts of Application--Filing Date Granted" dated **September 25, 1997**, a response to which is due **November 25, 1997**, please note that while the Serial Number that appears on the Notice to File Missing Parts is correct, the filing date and first named applicant is **incorrect**.

The correct filing date, as evidenced by a copy of the date-stamped return post card, is **April 11, 1997** (a copy of same is attached hereto). The subject application was filed by Express Mail Post Office and therefore is entitled to its mailing date of April 11, 1997 under 37 CFR §1.10.

10/16/1997 TTUTT1 00000041 08837009
01 FC:105 130.00 OP

The correct first named applicant is **Graeme I. Bell.** Dr. Bell's executed Declaration form was filed along with the application on April 11, 1997, along with the executed Declaration form of Dr. Merryweather.

Applicants respectfully request that the filing date and first named application be corrected. Enclosed herewith for filing is:

(XX) The original Combined Declaration and Power of Attorney, executed by second named inventor **Leslie B. Rall.** An unexecuted copy of this document, attached to the above-identified specification, was filed by Express Mail No. **EM347374321US** on **April 11, 1997.**

(XX) An Associate Power of Attorney is also enclosed.

( ) Small entity status of this application under 37 CFR 1.9 and 1.27 has been established by a Verified Statement previously submitted on _____.

( ) The original executed Verified Statement Claiming Small Entity Status Under 37 CFR 1.9 and 1.27 is filed herewith.

( ) A Verified Statement Claiming Small Entity Status Under 37 CFR 1.9 and 1.27 has been forwarded to the Refund Section, Accounting Division, Office of Finance on ___. A copy of this Verified Statement Claiming Small Entity Status form and the Request for Refund form is enclosed for your convenience.

(XX) A check in the amount of $ 130 is attached to cover the surcharge. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

( ) Please charge the surcharge to my Deposit Account No. 23-3050 in the amount of $____.

This sheet is attached in triplicate.

Date: October 2, 1997

Francis A. Paintin
Registration No. 19,386

WOODCOCK WASHBURN KURTZ
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DOCKET NO.: 2300.0202

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re reissue application of:

Graeme I. Bell et al.
U.S. Patent No. 5,405,942
Issued: April 11, 1995

Serial No.: Not yet assigned. Group No.: 1804

Filed: Herewith. Examiner: J. Stone

For: PREPRO INSULIN-LIKE GROWTH FACTORS I AND II

"Express Mail" Label No. EM347374321US
Date of Deposit 4/11/97

I hereby certify that this Reissue Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Typed Name: Bob Inforzato

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

REISSUE APPLICATION TRANSMITTAL LETTER

Transmitted herewith is the application for reissue of U.S. Patent No. 5,405,942 issued on April 11, 1995.

Enclosed are the following:

1. SPECIFICATION, CLAIM(S) AND DRAWING(S)

(XX) 6 page(s) of specification

(XX) 4 page(s) of claims

(XX) 1 page(s) of abstract

Note: This must include the entire specification and claims of the patent, with the matter to be omitted by reissue enclosed in square brackets; and any additions made by the reissue must be underlined, so that the old and new specifications and claims may be readily compared. Claims should not be renumbered and the numbering of claims added by reissue should follow the number of the highest numbered patent claim.

( ) _____ sheet(s) of ( ) formal/( ) informal drawings.

(XX) No changes in the drawings upon which the original

patent was issued are to be made. Therefore, in accordance with 37 C.F.R. § 1.174, please find attached, in the size required for original drawings:

(XX) a copy of the printed drawings of the patent.

( ) a photoprint of the original drawings.

2. DECLARATION AND POWER OF ATTORNEY

(XX) __7__ pages of declaration and power of attorney for each of inventors G.I. Bell and J.P. Merryweather; **the declaration of inventor L.B. Rall is not enclosed but will be filed within the time set under 37 CFR 1.53(d)(1) and MPEP §1414.**

( ) An Associate Power of Attorney.

3. PRELIMINARY AMENDMENT (check if applicable)

(XX) enclosed herewith.

4. OFFER TO SURRENDER THE ORIGINAL LETTERS PATENT IN ACCORDANCE WITH 37 C.F.R. § 1.178 IS ATTACHED

(XX) Offer to surrender is by the inventor.

(XX) along with Consent of Assignee and Certificate under 37 CFR §3.73(b).

( ) Offer to surrender is by the assignee of the entire interest (and the reissue application does not seek to enlarge the claims of the original patent).

5. LETTERS PATENT

( ) Original letters patent attached.

( ) Declaration that original letters patent lost or inaccessible.

(XX) Original letters patent or declaration that original letters patent lost or inaccessible will be submitted after prosecution on the merits but before the application has been allowed.

Note: "The application may be accepted for examination in the absence of the original patent or the declaration but one or the other must be supplied before the case is allowed." 37 C.F.R. § 1.178.

Note: "If a reissue be refused, the original patent will be returned to applicant upon his request." 37 C.F.R. § 1.178.

6. TITLE

In accordance with 37 C.F.R. § 1.171, this application for reissue is accompanied by:

( ) a certified copy of an abstract of title.

or

(XX) an order for an abstract of title.

7. INFORMATION DISCLOSURE STATEMENT (check if applicable)

( ) attached.

8. PRIORITY - 35 U.S.C. § 119

( ) Priority of application Serial No. _____ filed on _____ in _____ (country) is claimed under 35 U.S.C. § 119.

( ) The certified copy has been filed in prior application Serial No. _____ filed on _____.

9. FEE CALCULATION (37 C.F.R. §§ 1.16(h), (i) and (j))

CLAIMS AS FILED				SMALL ENTITY			OTHER THAN SMALL ENTITY	
	No. Filed		No. Extra	Rate	Fee	OR	Rate	Fee
BASIC FEE					$385	OR		$770
Total Claims	41	- 20 OR the number of claims in original patent, whichever is greater = 22	19	x $11=	$	OR	x $22=	$418
Indep. Claims	3	minus number of independent claims in original patent = 3	0	x $40=	$	OR	x $80=	$0
Multiple dependent claims are treated as ordinary claims for fee purposes. 37 C.F.R. § 1.16(j).								
TOTAL					$			1188

10. SMALL ENTITY STATUS (if applicable)

Note: A new verified statement is required for the reissue even if one has been filed in the original patent.

( ) A Verified Statement Claiming Small Entity Status under 37 C.F.R. §§ 1.9 and 1.27 is enclosed.

11. METHOD OF PAYMENT OF FEES

(XX) A check in the amount of $ 1188 is attached. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

DOCKET NO.: 2300.0202

Consent of Assignee

Chiron Corporation, assignee of the entire interest in Bell et al., U.S. Patent No. 5,405,942, hereby consents to the filing of the accompanying application for reissue of said patent.

Chiron Corporation

By _____

Name: Joseph H. Guth

Title: Assistant Secretary

Date: April 4, 1997

( ) Please charge my Deposit Account No. 23-3050 in the amount of $____.

This sheet is attached in triplicate.

12. **AUTHORIZATION TO CHARGE ADDITIONAL FEES**

(XX) The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 23-3050.

(XX) Any additional filing fees required under 37 C.F.R. §§ 1.16(a), (f) or (g) (filing fees) including fees for presentation of extra claims (37 C.F.R. §§ 1.16(b), (c) and (d)).

(XX) Any additional patent application processing fees under 37 C.F.R. § 1.17 and under 37 C.F.R. § 1.20(d).

(XX) The Commissioner is hereby authorized to charge payment of the following fees during the pendency of this application or credit any overpayment to Deposit Account No. 23-3050.

(XX) Any patent application processing fees under 37 C.F.R. § 1.17 and under 37 C.F.R. § 1.20(d).

( ) The issue fee set in 37 C.F.R. § 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b).

(XX) Any filing fees under 37 C.F.R. § 1.16 including fees for presentation of extra claims.

This sheet is attached in triplicate.

Date: April 9, 1997

Francis A. Paintin
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PREPRO INSULIN-LIKE GROWTH FACTORS I AND II

This application is a continuation of application Ser. No. 630,557, filed 13 Jul. 1984, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

It is suspected that somatic growth which follows the administration of growth hormones in vivo is mediated through a family of mitogenic, insulin-like peptides whose serum concentrations are growth hormone dependent. These polypeptides include somatomedin-C, somatomedin-A, and insulin-like growth factors I and II (IGF-I and IGF-II). IGF-I and IGF-II are single chain serum proteins of 70 and 67 amino acids, respectively, and there is evidence that they are identical to somatomedin-C and somatomedin-A. Although IGF-I and IGF-II can be isolated from human serum, such separation at best provides only limited quantities of the growth factors. It would thus be of great scientific and clinical interest to be able to produce relatively large quantities of the growth factors by recombinant DNA techniques. In order to do so, it is necessary to have DNA sequences which encode for IGF-I and IGF-II. In particular, it would be desirable to derive such DNA sequences from their natural source, i.e., human genetic information (RNA or DNA).

2. Description of the Prior Art

The amino acid sequences for human insulin-like growth factors I and II were first determined by Rinderknecht and Humbel (1978) J. Biol. Chem. 253:2769–2776 and Rinderknecht and Humbel (1978) FEBS. Lett. 89:283–286, respectively. The chemical synthesis of biologically active IGF-I has been reported. Li et al. (1983) Proc. Natl. Acad. Sci. USA 80:2216–2220. See also copending application Ser. No. 487,950, filed Apr. 25, 1983, which discloses the expression of synthetic genes for IGF-I and IGF-II in yeast.

SUMMARY OF THE INVENTION

Nucleotide sequences including both DNA and RNA are provided which code for human insulin-like growth factors (IGF) I and II and their corresponding polypeptide precursors. The DNA sequences may be used for production of the IGF and precursor polypeptides and biologically-active portions thereof in microorganisms or cell culture, while both the DNA and RNA sequences are useful as labelled probes in detecting the presence of the growth factor genes and/or mRNA sequences in a natural source. The nucleotide sequences of the present invention are derived from genetic information isolated from human cells, typically liver cells. In the exemplary embodiment, a cDNA library derived from human liver cells is screened with radiolabelled hybridization probes encoding a short nucleotide sequence common to both IGF-I and IGF-II. In this way, DNA sequences encoding for both preproIGF-I and preproIGF-II were detected and isolated.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 sets forth the nucleotide sequence derived from plasmid phigf1 encoding human preproIGF-I. The predicted amino acid sequence of the prepro protein is provided, and the first amino acid of the mature protein is designated as number 1. The region corre-

sponding to mature IGF-I is boxed, and pairs of basic amino acids are underlined.

FIG. 2 sets forth the nucleotide sequence derived from plasmid phigf2 encoding human preproIGF-II. The predicted amino acid sequence of the prepro protein is numbered with the first amino acid designated as number −24. The region corresponding to mature IGF-II is boxed, and pairs of basic amino acids are underlined.

FIG. 3 is a schematic representation of the structure of preproIGF-II. The proteolytic processing site of proIGF-II is indicated by an arrow; K and R denote lysine and arginine, respectively.

FIG. 4 is a schematic representation of the structure of preproIGF-I. The proteolytic processing site of proIGF-I is indicated by an arrow; K and R denote lysine and arginine, respectively.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

According to the subject invention, DNA and RNA sequences encoding for human IGF-I and IGF-II prepro polypeptides, or portions thereof, are provided. Such nucleotide sequences are useful for a variety of purposes. Both DNA and RNA sequences including at least 12 bases, more usually at least 18 bases, and frequently having 50 bases or more, can be used as hybridization probes for detecting complementary sequences in genomic DNA or in messenger RNA. Such probes may be used for detecting mutations and/or deletions in humans suspected of suffering from growth deficiencies. Longer DNA sequences may be used for expressing the precursor and/or mature proteins incorporating IGF, or any fragments or analogs thereof. Production of the precursor polypeptides will often be desirable since the precursor will be amenable to post-translational processing in appropriate hosts. The DNA sequences may also be used for the production of mRNA for any of the above purposes.

Both IGF-I and IGF-II are initially translated as "prepro" polypeptides including an amino-termino signal peptide and a carboxy-terminal peptide, referred to as the E domain. The signal peptide directs secretion of the prepro polypeptide across intracellular membranes and is cleaved during such secretion to form the "pro" polypeptide. Mature IGF-I and IGF-II are formed by subsequent proteolysis of the carboxy-terminal E domain from the pro polypeptide.

The nucleotide sequences of the present invention will be derived from human cells, typically by screening a human cDNA or genomic DNA library with hybridization probes capable of detecting a nucleotide sequence predicted from the known amino acid sequences of IGF-I and II. While suitable genomic libraries may be derived from human cells of any origin, it is preferred to utilize cDNA libraries from cells which are known to express the insulin-like growth factors, such as human liver cells and human fetal cells. Suitable hybridization probes may be synthesized by well known techniques and should employ degenerate coding to provide for all possible codons corresponding to each amino acid. In the exemplary embodiment, a human liver cDNA library developed by Woods et al. ((1982) Proc. Natl. Acad. Sci. USA 79:5661–5665) was screened with a 23 base oligonucleotide probe based on an 8 amino acid sequence common to both IGF-I and IGF-II.

The DNA sequences of interest in the present invention may be single or double stranded and will include at least about 12 bases, preferably 18 bases or more, for single stranded oligonucleotides useful as hybridization probes. Double stranded fragments used for expression of polypeptides will usually be longer, typically being at least 18 base pairs corresponding to a sequence of 6 amino acids, more typically being at least the length of the coding region for the mature polypeptide, or a physiologically active fragment thereof. The DNA sequences may extend the entire length of the coding region for the prepro polypeptide, and may include untranslated and/or untranscribed flanking regions on either side of such coding region and/or intervening sequences.

Once the IGF DNA of interest has been isolated from the human cellular source, it will usually be cloned and expanded to provide sufficient amounts of the DNA for the intended use. Once sufficient amounts of the DNA have been obtained, the DNA sequence may be modified in a number of ways. For example, DNA sequences used as hybridization probes will be cleaved to a desired length using restriction enzymes, denatured to single-stranded form, and labelled, typically with a radiolabel, to allow detection. For expression of the mature IGF polypeptides, it may be desirable to excise the coding regions for the mature polypeptide and insert such coding regions into a suitable expression vector. In this way, the mature polypeptide may be expressed in hosts which are incapable of processing the prepro or pro polypeptide. Alternatively, in suitable hosts it may be desirable to employ the coding region for the entire prepro polypeptide either with or without associated flanking or intervening sequences.

The DNA sequences of the present invention may be replicated and expressed in a wide variety of hosts, including prokaryotes, eukaryotes, and mammalian cell culture. The cDNA sequences may be introduced into the host by conventional techniques, usually employing an extrachromosomal element capable of stable replication within the host. Alternatively, the DNA may be introduced directly into the genomic DNA using, e.g., co-transformation as described by Wigler et al. (1979) Cell 16:777-785. Hosts of particular interest include unicellular microorganisms, such as *E. coli, S. cerevisiae,* and *B. subtilis.*

A wide variety of suitable extrachromosomal elements exist for the cloning and expression of the IGF DNA sequences of the present invention. The cloning vectors will be selected to include a replication system suitable for the intended host. Suitable expression vectors for mammalian cells are well known in the art and include those having replication systems derived from viral genomes or portions thereof, e.g., SV-40, retroviruses, and the like. Replication systems for *E. coli* include those derived from various plasmids, such as R6-5, ColE1, RSF, and the like. Particularly convenient is plasmid pBR322 which includes a replication system derived from pMV1. Vectors suitable for yeast include those having a replication system derived from the 2 μm plasmid, autonomously replicating sequences (ars), and the like. Frequently, it will be desirable to have replication systems for both *E. coli* and a higher organism, e.g., yeast, present on the same extrachromosomal element. Such vectors, referred to as shuttle vectors, allow for cloning and amplification of the IGF gene in bacteria, while expression may be achieved in the higher organism with appropriate RNA or post-transla-

tional processing, e.g., cleavage of the pro polypeptide at the appropriate site to yield the mature polypeptide, polyadenylation, splicing, and the like.

In addition to the replication system, suitable extrachromosomal elements will usually include at least one marker for each intended host cell which allows for selection or selective pressure to maintain the extrachromosomal element containing the IGF DNA sequence. Convenient markers include biocidal resistance, e.g., antibiotics, heavy metals and toxins; complementation in an auxotrophic host, and the like.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Methods

Transformants (approximately 9000) from the adult human liver cDNA library of Woods et al. (1982) Proc. Natl. Acad. Sci. USA 79:5661–5655 were grown in 96-well microtiter dishes. The cDNA library was constructed by inserting ds cDNA prepared from mRNA from adult human liver cells into the PstI restriction site of pKT218. Plasmid pKT218 is a pBR322 derivative described by Talmadge et al. (1980) Proc. Natl. Acad. Sci. USA 77:3369–3373. Colonies of transformed *E. coli* were transferred to Whatman 541 paper, grown, amplified with chloramphenicol, and lysed as described by Gergen et al. (1979) Nucleic Acids Res. 7:2115–2136. Colonies containing IGF sequences were identified by hybridization with a 256-fold degenerate 23 base oligonucleotide which had been labelled with [γ-^{32}P]-ATP and T4 polynucleotide kinase. The oligonucleotide was synthesized manually (Urdea et al. (1983) Proc. Natl. Acad. Sci. USA 80:7461–7465) and purified by electrophoresis in a 8M urea, 20% polyacrylamide gel. The filters were hybridized in 5XSSC (SSC is 0.15M NaCl, 0.015M sodium citrate), 50 mM sodium phosphate, pH 7.0, 0.2% sodium dodecyl sulfate (SDS), 2X Denhardt's (Denhardt (1966) Biochem. Biophys. Res. Commun. 23:641–646), 200 μg/ml, sonicated and denatured salmon testes DNA, and 10^6 cpm/ml of 32P-labelled oligonucleotide at 30° C. After 16 hr, the filters were washed in 5XSSC and 0.1% SDS at 42° C. for one hour. Hybridizing colonies were identified by autoradiography. The inserted DNA fragments in the plasmids from the hybridizing colonies were sequenced. The sequence of the fragments carrying IGF-I or IGF-II DNA were determined on both strands and across all restriction sites used to initiate sequence determinations by the procedures of Maxam and Gilbert supra. and Sanger et al. (9180) J. Mol. Biol. 143:161–178.

Results

The nucleotide sequence of the hybridization probe was based on an eight amino acid sequence common to the sequences of IGF-I (amino acids 46–53) and IGF-II (amino acids 45–52), as reported by Rinderknecht and Humbel (1978) J. Biol. Chem. 253:2769–2776 and FEBS Lett. 89:283–286. The sequence was as follows.

	Glu	Cys	Cys	Phe	Arg	Ser	Cys	Asp	
5'-	GAG	TGT	TGT	TTT	CGA	AGT	TGT	GA	-3'
	A	C	C	C	T	C	C		
					G				
					C				

Eight of the approximately 9000 colonies hybridized with this probe, and analyses of the inserted PstI fragments derived from the cloned plasmids revealed that

only four of the eight fragments were of different sizes. The nucleotide sequences of each of the four distinct fragments were determined, and the predicted amino acid sequences corresponding to each of the three reading frames compared with the known sequences of IGF-I and IGF-II. The fragments in two of the plasmids (designated phigf 1 and phigf 2) were found to encode IGF-I and IGF-II, respectively. The proteins encoded by the other fragments were not related to IGF.

The plasmid phigf 1 contained a PstI insert of approximately 660 base pairs (bp) which extended from the second nucleotide of the codon for amino acid −15 of the putative signal peptide of the preproIGF-I to the poly A tract and included about 245 bp of 3′ untranslated region and a variant polyadenylation signal AATAAT (residues 595–600). The nucleotide sequence and corresponding amino acid sequence are set forth in FIG. 1. The number of the nucleotide at the end of each line is indicated; the region corresponding to mature IGF-I is boxed and pairs of basic amino acids are underlined.

The nucleotide sequence of human preproIGF-II mRNA was deduced from the sequence of the inserted fragment in plasmid phigf 2. Referring to FIG. 2, the predicted amino acid sequence of preproIGF-II is numbered with the first amino acid of preproIGF-II designated as number −24. The region corresponding to mature IGF-II is boxed and pairs of basic amino acids are underlined. An 89 amino acid carboxy-terminal region comprises residues 68–156. The number of the nucleotide at the end of each line is indicated. The B-domain (FIG. 3) of IGF-II comprises residues 1–32, the C-domain comprises residues 33–40, the A-domain comprises residues 41–61, the D-domain comprises residues 62–67, and the carboxyl-terminal E-domain comprises residues 68–156. In comparison, the B-domain of IGF-I (FIG. 4) comprises residues 1–29, the C-domain comprises residues 30–41, the A-domain comprises residues 42–62, the D-domain comprises residues 63–70, and the carboxy-terminal E-domain comprises residues 71–105.

Translation of the IGF-II mRNA from the initial Met (nucleotides 251–253, FIG. 2) predicts an 180 amino acid protein in which the 67 amino acid sequence of IGF-II begins 25 residues from the start. Thus, including the opal termination codon, the coding region is 543 bases. The 5′-untranslated region of the mRNA is at least 250 bases, and the 3′-untranslated region is greater than 253 bases. The cDNA clone phigf 2 lacks a poly A tract and polyadenylation signal.

No other clones encoding preproIGF-II mRNA were revealed when the insert in phigf 2 was used as a probe to rescreen the original 9000 colonies and 6000 additional colonies. A similar experiment using the insert of phigf1 as a probe to screen the same 15,000 colonies revealed, besides phigf1, only a second, identical clone, previously detected in the original screen, and phigf2 which cross-hybridized weakly. Attempts to determine the sizes of human preproIGF-I and preproIGF-II mRNAs by hybridization of the inserts to a northern blot (Thomas (1980) Proc. Natl. Acad. Sci. USA 77:5201–5205) of human adult liver poly A+ RNA were inconclusive, presumably because of the low abundance of these mRNAs (<1/10,000 molecules) in this tissue.

Both IGF-I and IGF-II are secreted proteins, and the 24 residue amino-terminal extension of the latter ap-

pears to be the signal peptide. Analysis of the hydrophilicity of preproIGF-II (as described by Hopp and Woods (1981) Proc. Natl. Acad. Sci. USA 78:3824–3828) indicates that the putative signal peptide has a hydrophobic core of 14 residues (amino acids −15 to −2) and a profile similar to other signal peptides. It is concluded that the homologous amino-terminal extension of preproIGF-I also represents a signal peptide of at least 15 amino acids. Interestingly, about 25% of the purified human IGF-II molecules lack Ala 1 (Rinderknecht and Humbel (1978) FEBS Lett. 89:283–286) suggesting that cleavage of the Ala(−1)–Ala(1) peptide bond by the peptidase is preferred but that the Ala(1-)–Tyr(2) bond is also cleaved.

The ≧15 and 24 residue amino-terminal extensions are cleaved from preproIGF-I and preproIGF-II, respectively, to produce proIGF-I and proIGF-II. ProIGF-II includes the 89 amino acid carboxyl-terminal extension referred to as the E-domain, and proteolytic processing at Arg 68 is required to produce mature IGF-II. Similarly, proIGF-I contains an E domain but of only 35 amino acids with requisite proteolytic processing to produce mature IGF-I at Arg 71. This carboxyl-terminal extension also has a potential N-linked glycosylation site (residues 92–94:Asn-Ala-Ser), which is absent in the IGF-II precursor. Although proteolytic processing at single basic residues has been reported in the generation of other proteins, including epidermal growth factor (Scott et al. (1982) Science 221:236–240) and growth hormone releasing factor (Gubler et al. (1983) Proc. Natl. Acad. Sci. USA 80:4311–4314; Mayo et al. (1983) Nature 306:86–88), processing occurs more often at pairs of basic amino acids. Only two such sites occur in proIGF-I (indicated by underlining in FIG. 1), both of which are within the mature polypeptide (residues 36–37 and 55–56), and thus remain uncleaved. There are five paired basic amino acids in proIGF-II (underlined in FIG. 2) including one site within mature IGF-II (residues 37–38) that is not cleaved. It is unknown if proteolysis occurs at any of the other pairs of basic residues (proIGF-II) or single basic amino acids within either of the E-domains.

In accordance with the subject invention, polynucleotide sequences are provided which encode insulin-like growth factors I and II. The polynucleotide sequences are derived from human genetic information (either DNA or RNA), typically by screening a cDNA library with an appropriate hybridization probe, and are useful for expression of the prepro polypeptide as well as the mature polypeptide. Additionally, the cloned polynucleotides themselves may be labelled and used as hybridization probes for a variety of purposes, such as genetic screening.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A composition comprising nucleic acid molecules containing a human sequence encoding insulin-like growth factor (hIGF) substantially free of nucleic acid molecules not containing said hIGF sequence, wherein said hIGF sequence is selected from the group consisting of:

(a) 5'-GGA CCG GAG ACG CUC UGC GGG GCU GAG CUG GUG GAU GCU CUU CAG UUC GUG UGU GGA GAC AGG GGC UUU UAU UUC AAC AAG CCC ACA GGG UAU GGC UCC AGC AGU CGG AGG GCG CCU CAG ACA GGU AUC GUG GAU GAG UGC UGC UUC CGG AGC UGU GAU CUA AGG AGG CUG GAG AUG UAU UGC GCA CCC CUC AAG CCU GCC AAG UCA GCU-3', wherein U can also be T;

(b) 5'-GCU UAC CGC CCC AGU GAG ACC CUG UGC GGC GGG GAG CUG GUG GAC ACC CUC CAG UUC GUC UGU GGG GAC CGC GGC UUC UAC UUC AGC AGG CCC GCA AGC CGU GUG AGC CGU CGC AGC CGU GGC AUC GUU GAG GAG UGC UGU UUC CGC AGC UGU GAC CUG GCC CUC CUG GAG ACG UAC UGU GCU ACC CCC GCC AAG UCC GAG-3', wherein U can also be T;

(c) nucleic acid sequences complementary to (a) or (b); and

(d) fragments of (a), (b) or (c) that are at least 18 bases in length and which will selectively hybridize to human genomic DNA encoding hIGF.

2. A composition according to claim 1 wherein said hIGF is hIGF-I and said hIGF sequence is sequence (a).

3. A composition according to claim 1 wherein said hIGF is hIGF-II and said hIGF sequence is sequence (b).

4. A composition according to claim 2 wherein said nucleic acid molecules comprise the following sequence, wherein U can also be T:

5'-CUG GCG CUG UGC CUG CUC ACC UUC ACC AGC UCU GCC ACG GCU GGA CCG GAG ACG CUC UGC GGG GCU GAG CUG GUG GAU GCU CUU CAG UUC GUG UGU GGA GAC AGG GGC UUU UAU UUC AAC AAG CCC ACA GGG UAU GGC UCC AGC AGU CGG AGG GCG CCU CAG ACA GGU AUC GUG GAU GAG UGC UGC UUC CGG AGC UGU GAU CUA AGG AGG CUG GAG AUG UAU UGC GCA CCC CUC AAG CCU GCC AAG UCA GCU CGC UCU GUC CGU GCC CAG CGC CAC ACC GAC AUG CCC AAG ACC CAG AAG GAA GUA CAU UUG AAG AAC GCA AGU AGA GGG AGU GCA GGA AAC AAG AAC UAC AGG AUG-3'.

5. A composition according to claim 3 wherein said nucleic acid molecules comprise the following sequence, wherein U can also be T:

5'-AUG GGA AUC CCA AUG GGG AAG UCG AUG CUG GUG CUU CUC ACC UUC UUG GCC UUC GCC UCG UGC UGC AUU GCU GCU UAC CGC CCC AGU GAG ACC CUG UGC GGC GGG GAG CUG GUG GAC ACC CUC CAG UUC GUC UGU GGG GAC CGC GGC UUC UAC UUC AGC AGG CCC GCA AGC CGU GUG AGC CGU CGC AGC CGU GGC AUC GUU GAG GAG UGC UGU UUC CGC AGC UGU GAC CUG GCC CUC CUG

GAG ACG UAC UGU GCU ACC CCC GCC
AAG UCC GAG AGG GAC GUG UCG ACC
CCU CCG ACC GUG CUU CCG GAC AAC
UUC CCC AGA UAC CCC GUG GGC AAG
UUC UUC CAA UAU GAC ACC UGG AAG
CAG UCC ACC CAG CGC CUG CGC AGG
GGC CUG CCU GCC CUC CUG CGU GCC
CGC CGG GGU CAC GUG CUC GCC AAG
GAG CUC GAG GCG UUC AGG GAG GCC
AAA CGU CAC CGU CCC CUG AUU GCU
CUA CCC ACC CAA GAC CCC GCC CAC
GGG GGC GCC CCC CCA GAG AUG GCC
AGC AAU CGG AAG UGA-3'.

6. A composition according to claim 1 wherein said nucleic acid molecules are DNA.

7. A composition according to claim 1 wherein said nucleic acid molecules are RNA.

8. A composition comprising cellular hosts transformed by a heterologous DNA sequence substantially free of cellular hosts that do not contain said heterologous DNA sequence, wherein said heterologous DNA sequence is a human sequence encoding insulin-like growth factor (hIGF) selected from the group consisting of:

(a) 5'-GGA CCG GAG ACG CTC TGC GGG
GCT GAG CTG GTG GAT GCT CTT CAG
TTC GTG TGT GGA GAC AGG GGC TTT
TAT TTC AAC AAG CCC ACA GGG TAT
GGC TCC AGC AGT CGG AGG GCG CCT
CAG ACA GGT ATC GTG GAT GAG TGC
TGC TTC CGG AGC TGT GAT CTA AGG
AGG CTG GAG ATG TAT TGC GCA CCC
CTC AAG CCT GCC AAG TCA GCT-3';

(b) 5'-GCT TAC CGC CCC AGT GAG ACC CTG
TGC GGC GGG GAG CTG GTG GAC ACC
CTC CAG TTC GTC TGT GGG GAC CGC
GGC TTC TAC TTC AGC AGG CCC GCA
AGC CGT GTG AGC CGT CGC AGC CGT
GGC ATC GTT GAG GAG TGC TGT TTC
CGC AGC TGT GAC CTG GCC CTC CTG
GAG ACG TAC TGT GCT ACC CCC GCC
AAG TCC GAG-3';

(c) nucleic acid sequences complementary to (a) or (b); and

(d) fragments of (a), (b) or (c) that are at least 18 bases in length and which will selectively hybridize to human genomic DNA encoding hIGF.

9. A composition according to claim 8 wherein said heterologous DNA sequence is selected from the group consisting of (a), (b) and (c).

10. A composition according to claim 9 wherein said hIGF is hIGF-I and said heterologous DNA sequence is (a).

11. A composition according to claim 9 wherein said hIGF is hIGF-II and said heterologous DNA sequence is (b).

12. A composition according to claim 10 wherein said heterologous DNA sequence comprises the following sequence:

5'-CTG GCG CTG TGC CTG CTC ACC TTC
ACC AGC TCT GCC ACG GCT GGA CCG
GAG ACG CTC TGC GGG GCT GAG CTG
GTG GAT GCT CTT CAG TTC GTG TGT
GGA GAC AGG GGC TTT TAT TTC AAC
AAG CCC ACA GGG TAT GGC TCC AGC

AGT CGG AGG GCG CCT CAG ACA GGT ATC GTG GAT GAG TGC TGC TTC CGG AGC TGT GAT CTA AGG AGG CTG GAG ATG TAT TGC GCA CCC CTC AAG CCT GCC AAG TCA GCT CGC TCT GTC CGT GCC CAG CGC CAC ACC GAC ATG CCC AAG ACC CAG AAG GAA GTA CAT TTG AAG AAC GCA AGT AGA GGG AGT GCA GGA AAC AAG AAC TAC AGG ATG-3'.

13. A composition according to claim 11 wherein said heterologous DNA sequence comprises the following sequence:

5'-ATG GGA ATC CCA ATG GGG AAG TCG ATG CTG GTG CTT CTC ACC TTC TTG GCC TTC GCC TCG TGC TGC ATT GCT GCT TAC CGC CCC AGT GAG ACC CTG TGC GGC GGG GAG CTG GTG GAC ACC CTC CAG TTC GTC TGT GGG GAC CGC GGC TTC TAC TTC AGC AGG CCC GCA AGC CGT GTG AGC CGT CGC AGC CGT GGC ATC GTT GAG GAG TGC TGT TTC CGC AGC TGT GAC CTG GCC CTC CTG GAG ACG TAC TGT GCT ACC CCC GCC AAG TCC GAG AGG GAC GTG TCG ACC CCT CCG ACC GTG CTT CCG GAC AAC TTC CCC AGA TAC CCC GTG GGC AAG TTC TTC CAA TAT GAC ACC TGG AAG CAG TCC ACC CAG CGC CTG CGC AGG GGC CTG CCT GCC CTC CTG CGT GCC CGC CGG GGT CAC GTG CTC GCC AAG GAG CTC GAG GCG TTC AGG GAG GCC AAA CGT CAC CGT CCC CTG ATT GCT CTA CCC ACC CAA GAC CCC GCC CAC GGG GGC GCC CCC CCA GAG ATG GCC AGC AAT CGG AAG TGA-3'.

14. A composition according to claim 9 wherein said heterologous DNA sequence is located on a plasmid that replicates in said cellular host.

15. A composition according to claim 9 wherein said cellular host is yeast.

16. A composition according to claim 9 wherein said cellular host is *E. coli*.

17. A composition according to claim 9 wherein said cellular host is *B. subtilis*.

18. A composition consisting essentially of nucleic acid molecules containing a human sequence encoding insulin-like growth factor (hIGF) selected from the group consisting of:

(a) 5'-GGA CCG GAG ACG CUC UGC GGG GCU GAG CUG GUG GAU GCU CUU CAG UUC GUG UGU GGA GAC AGG GGC UUU UAU UUC AAC AAG CCC ACA GGG UAU GGC UCC AGC AGU CGG AGG GCG CCU CAG ACA GGU AUC GUG GAU GAG UGC UGC UUC CGG AGC UGU GAU CUA AGG AGG CUG GAG AUG UAU UGC GCA CCC CUC AAG CCU GCC AAG UCA GCU-3', wherein U can also be T;

(b) 5'-GCU UAC CGC CCC AGU GAG ACC CUG UGC GGC GGG GAG CUG GUG GAC ACC CUC CAG UUC GUC UGU GGG GAC CGC GGC UUC UAC UUC AGC AGG CCC GCA

AGC CGU GUG AGC CGU CGC AGC CGU GGC AUC GUU GAG GAG UGC UGU UUC CGC AGC UGU GAC CUG GCC CUC CUG GAG ACG UAC UGU GCU ACC CCC GCC AAG UCC GAG-3', wherein U can also be T;

(c) nucleic acid sequences complementary to (a) or (b); and

(d) fragments of (a), (b) or (c) that are at least 18 bases in length and which will selectively hybridize to human genomic DNA encoding hIGF.

19. A composition according to claim 9 wherein said cellular host is *E. coli* HB101(phigf1).

20. A composition according to claim 1 wherein said nucleic acid molecules are phigf1.

21. A composition according to claim 9 wherein said cellular host is *E. coli* HB101(phigf2).

22. A composition according to claim 1 wherein said nucleic acid molecules are phigf2.

* * * * *

DOCKET No.: 2300.0202 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue application of:

Graeme I. Bell, et al.
U.S. Patent No. 5,405,942
Issued: April 11, 1995

Serial No.: unassigned Group Art Unit: 1804

Filed: herewith Examiner: J. Stone

For: PREPRO INSULIN-LIKE GROWTH FACTORS I AND II

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

DECLARATION AND POWER OF ATTORNEY
IN APPLICATION FOR REISSUE OF
BELL ET AL. U.S. PATENT No. 5,405,942

In accordance with the provisions of 35 USC §251 and 37 CFR §1.172(a), Graeme I. Bell, Leslie B. Rall and James P. Merryweather (whose citizenship, residence and post office address are set forth below), named as the original, first and joint inventors of the invention described and claimed in U.S. Patent No. 5,405,942 ("the original patent"), filed as Serial No. 07/065,673, on June 16, 1987, and granted on April 11, 1995, hereby apply for and assent to reissue of said patent and declare as follows:

1. We have reviewed and understood the entire specification and claims of the attached reissue application, and the accompanying Preliminary Amendment and we believe that we are the original, first and joint inventors of the subject matter

which is claimed and for which a reissue of said patent is being sought.

2. The duty to disclose to the Office all information known to said assignee and said inventors to be material to patentability as defined in 37 CFR §1.56 is hereby acknowledged.

3. It is verily believed that said original patent may be partly inoperative under 35 USC §251 for having claimed less than the inventors had a right to claim as explained in detail hereinafter.

4. This reissue application seeks to enlarge the scope of the claims of the original U.S. Patent No. 5,405,942.

5. Said original patent issued with claims 1 through 22 therein. None of those original claims is directed to any method, much less a method of producing a polypeptide, or of expression in a transformed host cell. The failure to claim such a method is an insufficiency of the original claims which is sought to be remedied by this reissue application by the addition of claims 23 through 41 in the accompanying Preliminary Amendment.

6. We have been advised and believe that the statements set forth in the following paragraphs 7-12 are true.

7. Applicants' application Serial No. 07/065,673, filed June 16, 1987, and its parent application Serial No. 06/630,557, filed July 13, 1984, never contained any type of method or process claims.

8. As seen from the foregoing, no method claims of the type now being sought were presented in Serial No. 07/065,673, at the time of filing on June 16, 1987, or during its pendency prior to issue of the original patent on April 11, 1995. It was error without deceptive intent on the part of applicants and assignee, that arose during that time period, to fail to present such method claims and to permit its original U.S. Patent No. 5,405,942 to issue without such claims.

9. The error described in paragraph 8 above was discovered by the assignee in the October-November 1996 period when the subject patent was evaluated for its potential exclusionary scope. At that time, it was noted that the absence of method or process claims in the subject patent would prevent the assignee from availing itself of the provisions of 35 USC §271(g) as to imports made abroad by a patented method or process. However, it was not then known for certain whether or not there were likely to be such imports for which exclusion was necessary.

10. Shortly after said error was discovered, assignee asked its attorneys in November 1996 to evaluate the patent to determine whether the error was correctable by filing a reissue to include the appropriate method claims therein. After thorough study and consultation, its attorneys advised that the error should be correctable by reissue.

11. After learning that the error should be correctable, and in view of the fact that the exisiting claims in the original patent were not adversely affected by the error, applicants' assignee undertook a study to determine if the error is significant enough to submit the patent to reissue examination to avail itself of the protection of 35 USC §271(g) in view of the potentially infringing activities of others. It was only recently that sufficient facts were learned showing that the error in not having the method claims in the patent is significant enough to merit this application for reissue, and that there may be imports for which protection is needed.

12. After reviewing the matter, we believe that through error and without deceptive intent applicants and assignee failed to recognize the error at the time it was made. We believe that the error is one correctable by filing the instant reissue application at this time with the newly added method claims 23-41 being added by the accompanying Preliminary Amendment in accordance with MPEP §1453.

13. Applicants declare that they have read and understood claims 1-22 of their original patent, and have read and understood claims 23-41 of said Preliminary Amendment and the Remarks made therein; applicants believe that they are the original, first and joint inventors of the invention claimed in claims 1-41 and which is described in their original patent.

13. Applicants and the assignee hereby offer to surrender the original U.S. Patent No. 5,405,942 in accordance with 37 CFR §1.178.

POWER OF ATTORNEY

Applicants hereby appoint Robert P. Blackburn, (Registration No. 30,447), Barbara G, McClung (Registration No. 33,113), Joseph A. Guth (Registration No. 31,261), Francis A. Paintin (Registration No. 19,386), Dianne B. Elderkin (Registration No.28,598), Doreen Y. Trujillo (Registration No. 35,719), as its attorneys with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith.

Send all future correspondence and address all telephone calls to:

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Law Department
4560 Horton Street
Emeryville, CA 94608-2916
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Facsimile (510) 655 3542

DECLARATION

We, the undersigned, hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued hereon.

DOCKET No.: 2300.0202 PATENT

Execution by Inventors:

Signed: _____ Date: _____
Graeme I. Bell

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(city and state)

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Chicago, IL Zip Code: 60637

Signed: _____ Date: _____
Leslie B. Rall

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P.O. Address: 222 Dellbrook Avenue

San Francisco, CA Zip Code 94131

Signed: James P. Merryweather Date: 4/8/97
James P. Merryweather

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3-00

DOCKET No.: 2300.0202 PATENT

1-00

Execution by Inventors:

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Chicago, IL Zip Code: 60637

2-00

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P.O. Address: 222 Dellbrook Avenue

San Francisco, CA Zip Code: 94131

Signed: _____ Date: _____

James P. Merryweather

Residence: _____ Citizenship: _____
(city and state)

P.O. Address: _____

_____ Zip Code _____

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue application of:

Graeme I. Bell, et al.
U.S. Patent No. 5,405,942
Issued: April 11, 1995

Serial No.: unassigned Group Art Unit: 1804

Filed: herewith Examiner: J. Stone

For: PREPRO INSULIN-LIKE GROWTH FACTORS I AND II

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

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IN APPLICATION FOR REISSUE OF
BELL ET AL. U.S. PATENT No. 5,405,942

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1. We have reviewed and understood the entire specification and claims of the attached reissue application, and the accompanying Preliminary Amendment and we believe that we are the original, first and joint inventors of the subject matter

which is claimed and for which a reissue of said patent is being sought.

2. The duty to disclose to the Office all information known to said assignee and said inventors to be material to patentability as defined in 37 CFR §1.56 is hereby acknowledged.

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11. After learning that the error should be correctable, and in view of the fact that the exisiting claims in the original patent were not adversely affected by the error, applicants' assignee undertook a study to determine if the error is significant enough to submit the patent to reissue examination to avail itself of the protection of 35 USC §271(g) in view of the potentially infringing activities of others. It was only recently that sufficient facts were learned showing that the error in not having the method claims in the patent is significant enough to merit this application for reissue, and that there may be imports for which protection is needed.

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POWER OF ATTORNEY

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Send all future correspondence and address all telephone calls to:

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Law Department
4560 Horton Street
Emeryville, CA 94608-2916
Telephone (510) 923 2700
Facsimile (510) 655 3542

DECLARATION

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DOCKET NO.: 2300.0202 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue application of:

Graeme I. Bell, et al.
U.S. Patent No. 5,405,942
Issued: April 11, 1995

Serial No.: unassigned Group Art Unit: 1804

Filed: herewith Examiner: J. Stone

For: PREPRO INSULIN-LIKE
GROWTH FACTORS I AND II

CERTIFICATE UNDER 37 C.F.R. §3.73(b) AND CONSENT OF ASSIGNEE

Chiron Corporation, of Emeryville, California, certifies that it is the assignee of the entire right, title and interest in the above-identified accompanying application for reissue by virtue of the following: an assignment from the inventors Graeme I. Bell, Leslie B. Rall and James P. Merryweather recorded in the U.S. Patent and Trademark Office at Reel 4306, Frame 0018. Copies of documents evidencing such assignment are enclosed herewith.

Assignee states that the evidentiary documents have been reviewed and certifies that, to the best of assignee's knowledge and belief, title is in the assignee taking action herein.